UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

KAIO DOS SANTOS

INFLUÊNCIA DO CAFÉ NA DINÂMICA DO REPARO ÓSSEO ALVEOLAR EM RATOS:
ANÁLISE DE MICROSCOPIA CONFOCAL A LASER

ALVEOLAR BONE REPAIR DYNAMICS IN RATS INFLUENCED BY THE COFFEE INGESTION:
LASER CONFOCAL MICROSCOPY ANALYSIS

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Trabalho de Conclusão de Curso apresentado à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Cirurgião Dentista.

Undergraduate final work presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Dental Surgeon

Orientador: Prof(a). Dr(a). ANA CLÁUDIA ROSSI

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RESUMO

O objetivo deste estudo foi investigar o efeito da ingestão de café na dinâmica óssea alveolar após extração dentária usando microscopia confocal a laser. Foram utilizados 8 ratos machos, linhagem Wistar, com 2 meses, que foram distribuídos em dois grupos, o Grupo Controle, em que foi mantida a dieta normal (água), e o Grupo Experimental com ingestão de café. Em ambos os grupos foi realizada a exodontia do incisivo superior direito. O corante fluorescente calceína foi injetado aos 14 dias e o corante fluorescente alizarina aos 28 dias nos ratos (ambos 25 mg/kg). A eutanásia da amostra ocorreu 28 dias após o dia da exodontia. A maxila direita foi removida, processada e analisada em microscópio confocal a laser. Com as imagens obtidas, foram obtidas a taxa de aposição mineral e a área de osso alveolar marcada com calceína e alizarina. Não houve diferença estatística significativa entre os grupos para a taxa de mineralização óssea (teste Mann Whitney, P=0,7756). Dados foram analisados com two-way ANOVA para comparar as diferenças entre os grupos (controle e café) e corantes fluorescentes (calceína e alizarina); a interação entre os grupos não foi estatisticamente significativa, já a interação entre os corantes foi (P<0,0001). A análise intragrupo mostrou diferença estatística significativa entre os corantes injetados aos 14 dias (calceína) e aos 28 dias (alizarina) após a extração dental (teste de Tukey P<0,0001). Como conclusão, houve uma tendência de diminuição de osso renovado no grupo que ingeriu café, apesar de não ter sido significativa.

Palavras-chave: Rato. Osso alveolar. Café. Corantes Fluorescentes.

ABSTRACT

The aim of this study was to investigate the effect of coffee intake on alveolar bone dynamics after tooth extraction using confocal laser microscopy. Eight male Wistar rats, aged 2 months, were used and divided into two groups, the Control Group, in which the normal diet (water) was maintained, and the Experimental Group with coffee ingestion. In both groups, extraction of the upper right incisor was performed. The fluorescent dye calcein was injected at 14 days and the fluorescent dye alizarin at 28 days in rats (both 25 mg/kg). The sample was euthanized 28 days after the day of extraction. The right maxilla was removed, processed and analyzed under a confocal laser microscope. With the images obtained, the mineral apposition rate and the alveolar bone area marked with calcein and alizarin were obtained. There was no statistically significant difference between groups for bone mineralization rate (Mann Whitney test, P=0.7756). Data were analyzed with two-way ANOVA to compare differences between groups (control and coffee) and fluorescent dyes (calcein and alizarin); the interaction between the groups was not statistically significant, whereas the interaction between the dyes was (P<0.0001). The intragroup analysis showed a statistically significant difference between dyes injected at 14 days (calcein) and at 28 days (alizarin) after tooth extraction (Tukey test P<0.0001). In conclusion, there was a trend towards a decrease in renewed bone in the group that drank coffee, although this was not significant.

Key words: Rat. Alveolar boné. Coffee. Fluorescent Dyes.

SUMÁRIO

1 INTRODUÇÃO

O osso em um estado de saúde está em constante reabsorção e deposição, de forma equilibrada, a fim de manter a integridade estrutural do tecido. Para isso, as células atuam juntas na sinalização para manter o turnover. Com o objetivo de compreensão da cicatrização do osso alveolar já foram feitos estudos pré-clínicos analisando o osso alveolar após a extração em condições clínicas e experimentais. Um modelo clássico é a extração de incisivos superiores em ratos descrevendo a cicatrização alveolar em três fases: formação de coágulo e proliferação celular a partir do tecido conjuntivo; formação de tecido conjuntivo e cicatrização; fase de ossificação (Hassumi et al., 2018). O reparo ósseo alveolar implica em uma série de reações teciduais que ocorrem no interior do alvéolo, após a exodontia, com o objetivo de preencher o alvéolo dental por tecido ósseo (Alves et al., 1989).

A cafeína está muito presente na dieta em diferentes fontes alimentares como café, chocolate e bebidas derivadas da cola. Além dos alimentos, a cafeína é encontrada em inúmeros medicamentos, inclusive os que não requerem uma prescrição médica. A cafeína é uma xantina metil que possui uma variedade de respostas celulares e farmacológicas, que induz a diversos efeitos biológicos como anti-oxidação, anti-mutação, angiogênica, ação antibiótica, anti-hipercolesterolemia, anti-hipertensivo e antiinflamatório (Macedo et al., 2015). A cicatrização e reparo dos tecidos possuem fatores que podem afetar positivamente ou negativamente este processo. No caso, o componente alimentar, ou seja, a dieta é o fator a ser estudado e que atua como componente crítico em todos os processos de cicatrização de feridas (Demling., 2009). Estudos com ratos mostraram que, de acordo com sua dieta, estes poderiam possuir uma menor carga e rigidez óssea máxima (Yamanaka et al., 2018).

Trabalhos anteriores mostraram que ratos em crescimento que tinham em sua dieta a cafeína possuíam diminuição da mineralização da matriz de suas tíbias. A cafeína causou reduções significativas na produção da matriz extracelular, mineralização e atividade da fosfatase alcalina, acompanhada de diminuição na expressão gênica das proteínas da matriz específica da cartilagem, como agrecano, colágeno tipo II e tipo X (Choi et al., 2017). Estudos também mostraram efeitos prejudiciais da cafeína no tecido ósseo causando impacto no equilíbrio de cálcio (Tassinari et al. 1991; Tsuang et al. 2006; Rapuri et al. 2007; Lu et al. 2008). Ainda se faz necessário estudos que avaliem a influência da cafeína na densidade de trabéculas ósseas após a fase do reparo ósseo alveolar.

Sobre o reparo alveolar, há um trabalho realizado em 2015 que mostrou que a ingestão diária de café fervido e a administração de cafeína pura afetaram o processo de reparo ósseo após a extração dentária em ratos, incluindo retardo na produção de tecido de

granulação (Macedo et al., 2015). Contudo, não há estudos que mostram o impacto do consumo de café diário na arquitetura das trabéculas ósseas e sua densidade e como ocorre a osteogênese após o reparo.

Estudos clínicos apresentam resultados conflitantes em relação a cafeína e ao cálcio uma vez que um estudo mostrou uma influência negativa na retenção do cálcio (Massey et al., 1993), no entanto outro trabalho feito demonstrou nenhuma relação entre a ingestão de cafeína e o metabolismo do cálcio (Cooper et al., 1992). Marcadores de fluorocromo já foram utilizados em trabalhos com ratos para detectar e medir atividades osteogênicas (Merzel et al., 2008). Fluorocromos são compostos químicos que possuem a capacidade de se ligar ao cálcio no momento da precipitação na matriz óssea orgânica. Portanto, as marcações de fluorocromo representam a quantidade de precipitação de cálcio, permitindo assim medir a formação óssea (Ramalho-Ferreira et al., 2015).

No presente trabalho, através de marcadores de fluorocromo analisamos o impacto do café na osteogênese após o reparo, uma vez que os estudos clínicos apresentam resultados conflitantes em relação ao metabolismo do cálcio, já que este mineral está diretamente ligado a mineralização da matriz óssea.

2 ARTIGO: ALVEOLAR BONE REPAIR DYNAMICS IN RATS INFLUENCED BY THE COFFEE INGESTION: CONFOCAL MICROSCOPY ANALYSIS

Submetido no periódico Clinical Oral Investigations (Anexo 4)

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Abstract

Objectives: to investigate the effect of coffee ingestion on alveolar bone dynamics after tooth extraction using confocal laser microscopy.

Materials and Methods: Eight male Wistar rats, aged 2 months, were used and divided into two groups, the Control Group, in which the normal diet (water) was maintained, and the Experimental Group with coffee ingestion. In both groups, extraction of the upper right incisor was performed. The fluorochrome calcein was injected at 14 days and the fluorochrome alizarin at 28 days in rats (both 25 mg/kg). The sample was euthanized 28 days after the day of extraction. The right maxilla was removed, processed and analyzed under a confocal laser microscope. With the images obtained, the mineral apposition rate and the alveolar bone area marked with calcein and alizarin were obtained.

Results: There was no statistically significant difference between groups for bone mineralization rate (Mann Whitney test, P=0.7756). Data were analyzed with two-way ANOVA to compare differences between groups (control and coffee) and fluorochromes (calcein and alizarin); the interaction between the groups was not statistically significant, whereas the interaction between the fluorochromes was (P<0.0001). The intragroup analysis showed a statistically significant difference between the fluorochromes injected at 14 days (calcein) and at 28 days (alizarin) after tooth extraction (Tukey test P<0.0001).

Conclusions: There was a tendency for there to be a decrease in renewed bone in the group that drank coffee, although this was not significant.

Clinical Relevance: to contribute to the dental field through knowledge of the influence of coffee on alveolar bone repair after tooth extraction, allowing the targeting of treatments, for example, with dental implants, in which the presence of a healthy bone and biomechanically active is required.

Keywords: Rat, Alveolar bone, Coffee, Fluorescent Dyes.

Introduction

Alveolar bone repair is a dynamic process that involves a series of tissue reactions that occur inside the alveolus, after tooth extraction, with the objective of filling the dental alveolus with bone tissue [1-3]. The process of alveolar bone healing in rats is fairly established in the literature [4]. Preclinical studies have been carried out analyzing the alveolar bone after extraction under clinical and experimental conditions, and it is known that its process is completed by 28 days after the day of tooth extraction [1, 5]. Só et al. [4] reported that, when it comes to alveolar bone healing analysis, rats are the most frequent animal model used, and the upper incisors and maxillary molars are the most frequent extracted teeth. A classic model is the extraction of upper incisors in rats describing alveolar healing in three phases: clot formation and cell proliferation from the connective tissue; connective tissue formation and scarring; ossification phase [1].

After tooth surgical extraction, it is necessary to maintain the environment of the oral cavity in appropriate conditions to favor alveolar bone repair. Healing and tissue repair have factors that can positively or negatively affect this process. The potential for bone healing is influenced by several mechanisms and factors, such as ageing, hormonal, biochemical and biomechanical ones [6-7]. Moreover, the diet and its components act as a critical component

in all wound healing processes, once it can exert influence over the ideal conditions, to favor or not alveolar bone repair [8-9]. This happens through the management of the behavior and function of the cells accountable for the formation of new bone [6].

Coffee includes multiple bioactive compounds, some with potentially therapeutic antioxidant, anti-inflammatory, antifibrotic, or anticancer effects [10-12]. Some of these compounds can affect bone metabolism [12]. Substances, such as antioxidants, may provide protection to bones, and are beneficial for health, while other specific ones may increase bone resorption [12-13]. Despite research into this field, there is inconsistency in the association between coffee consumption and musculoskeletal outcomes, and the impact of coffee consumption on bone metabolism remains controversial [11-12].

Regarding alveolar bone repair, there is a study carried out in 2015 that showed the daily intake of boiled coffee, and the administration of pure caffeine affected the bone repair process after tooth extraction in rats, including a delay in the production of granulation tissue [14]. However, there are no studies showing the impact of daily coffee consumption on the alveolar bone osteogenesis.

Fluorochrome markers have already been used in studies with rats to detect and measure osteogenic activities [15-16]. Fluorochromes are chemical compounds that can bind calcium at the time of precipitation in the organic bone matrix. Therefore, the fluorochrome markings represent the amount of calcium precipitation, thus allowing the measurement of bone formation [17]. This marker allows to assess a possible alteration caused by coffee at the time of osteogenesis in alveolar repair.

In the present study, through fluorochrome markers it was possible to analyze whether there is any impact on post-repair osteogenesis, since clinical studies present conflicting results in relation to calcium metabolism, and this mineral is directly linked to bone matrix mineralization. Thus, it is expected to contribute to the dental field through knowledge of the influence of coffee on alveolar bone repair after tooth extraction, allowing the targeting of treatments, for example, with dental implants, in which the presence of a healthy bone and biomechanically active is required. The aim of the study was to investigate the effect of coffee ingestion on alveolar bone dynamics after tooth extraction using confocal laser microscopy.

Material and methods

This study was approved by the Ethics Committee on Animal Experimentation (CEUA) from the Biology Institute (IB) of the University of Campinas (UNICAMP) (protocol number: 5324-1/2019).

Experimental design

Eight male Wistar rats (Rattus norvergicus albinus), 2 months old and weighing between 200 and 250g were used. The animals in the control group were kept in collective cages (4 animals/box), and the animals in the group with coffee ingestion were kept in individual cages, with a temperature of 22 ± 2ºC, controlled light cycle (12/12h) and access free to water and feed until two months of age. After this period, the control group continued to have free access to water and feed, but the group with coffee ingestion had controlled access to water and free feed.

The rats were randomly distributed into two groups for the experiments:

A) Control group: the rats received free access to water before and after tooth extraction surgery (n=4);

B) Coffee group: the rats received free access to water before tooth extraction surgery; the rats received water and coffee after tooth extraction surgery (n=4);

The Figure 1 showed the sequence of the events in the present study.

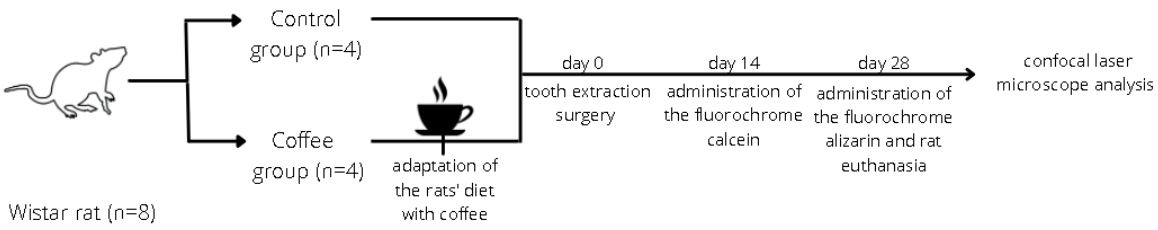

Fig. 1 Chronological flowchart of the events in this study

Adaptation model to the introduction of coffee

The amount of coffee ingested by the animals was estimated based on the daily human consumption of 4 cups (240 mL) per day for a person weighing 60 kg [14]. Thus, the

rats started to receive roasted, ground and cooked coffee (Morro Grande Coffee, Piracicaba, São Paulo, Brazil) for the adaptation of 50 mg / mL (1.2 mL of coffee infusion / day), reducing the supply of water, during the 28 days from the day zero of tooth extraction. The rats of the coffee group were placed in cages, separately (one per cage), to control the coffee ingestion.

Tooth extraction surgery

The procedure was performed under sedation via an intraperitoneal injection of ketamine (40-87 mg/kg) (Ketamin Chloridrate, injectable, Fort Dodge, Saúde Animal, Ltda), to promote anesthesia, and xylazine (5-13 mg/kg) (Xilazine-Coopers, Brazil, Ltda), to promote muscle relaxation. Then, antisepsis with polyvinylpyrrolidone iodide (Indústria Química e Farmacêutica Rioquímica Ltda, Brazil) was performed, and the right upper incisor was extracted using specific and adapted instruments [18]. The gingival mucosa was sutured with polyglactin 910 (Vicryl 4.0 – Jhonson & Jhonson, New Brunswick, NJ, USA). After the surgery procedure, was promote analgesia using an injection of ketoprofen (NSAID - 5 mg / kg) via subcutaneous, one time, per one day.

Euthanasia

The rats from both groups were euthanized at 28 days after day zero from tooth extraction surgery using excessive anesthetic dose. The head was disjointed of the body and dissected to obtain the skull and fixed in 10% formalin solution and 0.1M phosphate buffer (pH 7.4) for 24h at 4 ° C.

Laboratory procedures

Using an increasing sequence of alcohols (70 to 100%) the dissected maxilla was dehydrated. After the dehydration process, the solutions were soaked in methyl methacrylate (MMAL) solutions (Classic, Classic Dental Articles, São Paulo, SP, Brazil) in three baths to fix the pieces. The catalyst used was benzoyl peroxide (1%, Riedel-de Haën AG, Seelze-Hannover, Germany) added only in the last bath [2]. To obtain complete polymerization, the pieces were kept in test tubes inside an oven at 37º C for 5 days. After polymerization, the resin blocks were cut in the sagittal plane with the aid of a Maxicut mounted on a bench motor (Kota – São Paulo – SP, Brazil). The parts were then rubbed bilaterally with 120, 300, 400, 600, 800 and 1200 crescent grains mounted in an automatic polisher (ECOMET 250PRO / AUTOMET 250, Buehler, Lake Bluff, Illinois) until the cuts reached a thickness of 80 µm. For the measurement, a digital caliper was used (Mitutoyo, Pompeia, SP, Brazil). The slices were mounted in glass and mineral oil slides (liquid oil, Mantecor, Taquara, RJ, Brazil) and sealed

with a glass lid and enamel transparent to prevent oil leakage and possible dehydration of the samples [17].

Confocal Laser Microscopy

In both control and coffee groups, 2ug/kg body weight of intravenous green calcein was administered (Sigma Chemical Co., St. Louis, Mo, USA) at 14 days from the day of the extraction was performed. Calcein was prepared immediately before use. Each animal received in doses of 1mL of dye volume. In both groups, 2.5ug/kg body weight of intravenous alizarin red was administered (Sigma Chemical Co., St Louis, Mo, USA) 24 days from the day it was performed the extraction. Alizarin was prepared immediately before use. Each animal received doses of 1mL of dye volume.

Longitudinal sections of the area of interest (bone adjacent to the apical third of the right upper incisor) were obtained using a Leica TCS SP5 microscope (Leica Microsystems, Heidelberg, Germany) through a 10x objective (original magnification 100x). Thus, the area of the bone around this tooth was evaluated in each specimen.

The images obtained via confocal laser microscopy were reconstructed through the software grid installed to manipulate the microscope (Leica TCS SP5, Leica Microsystems, Heidelberg, Germany). The images obtained showed two colors representing calcium precipitation after the administration of calcein (green) and alizarin (red) at 28 days after extraction. The software overlay the images and presented both overlapping fluorochromes. The prominence of green color represented a greater amount of an older bone, whereas a red exaltation represented a greater amount of a younger bone [2, 17].

Bone area analysis

These images were saved in TIFF format and transported to ImageJ software (Processing Software and Image Analysis, Ontario, Canada). Using the known scale of each slide, the software measurements were calibrated. With the "color threshold" tool, each image was standardized according to hue, saturation, and brightness to reveal fluorochromes. The principle, the "free hands" tool, was selected; the calcein was highlighted; and the "measure" tool was used to calculate the bone area in μm2. The same procedure was performed with alizarin, with data related to the dynamics of the bone tissue in the region being obtained [2, 17].

Mineral apposition rate

To obtain the mineral apposition rate of the fluorochrome label, images containing the alizarin and calcein overlay were imported into Image J software (Processing Software and Image Analysis, Ontario, Canada). Choosing five regions randomly in each image, the "straight" tool was used, and a line was drawn from the beginning of alizarin precipitation (red label) to the end of calcein precipitation (green label). The mean values were divided by 14, representing the period between both fluorochrome injections, leading to the mineral apposition rate value [2, 19].

Statistical analysis

Data from mineral apposition rate were evaluated Mann Whitney test (Two-tailed). Data from bone area were analyzed with two-way analysis of variance (ANOVA). Significant results were statistically evaluated with Sidak's multiple comparisons test. All tests showed a significance level of 5%. The statistical program GraphPAD Prism v.8 (San Diego, CA, USA) was used.

Results

There was no statistically significant difference between groups for mineral apposition rate (Mann Whitney test, P=0.7756) (Figure 2).

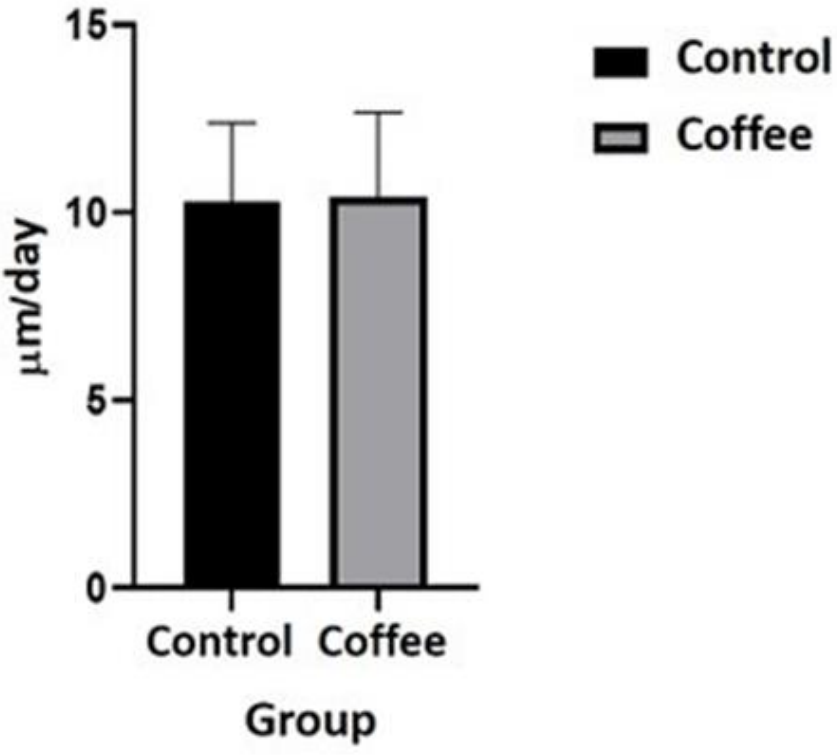

Fig. 2 Mineral apposition rate per day (μm) of the Coffee and Control groups, evaluated linearly. Results represented by mean, error bar by S.EM.

Data were analyzed with two-way ANOVA to compare differences between groups (control and coffee) and fluorochromes (calcein and alizarin). The interaction between the groups was not statistically significant, whereas the interaction between the fluorochromes was ($P<0.0001$). The intragroup analysis showed a statistically significant difference between the fluorochromes injected at 14 days (calcein) and at 28 days (alizarin) after tooth extraction (Tukey test $P<0.0001$) (Figure 3). In the coffee group, there was a tendency to have a decrease in alizarin.

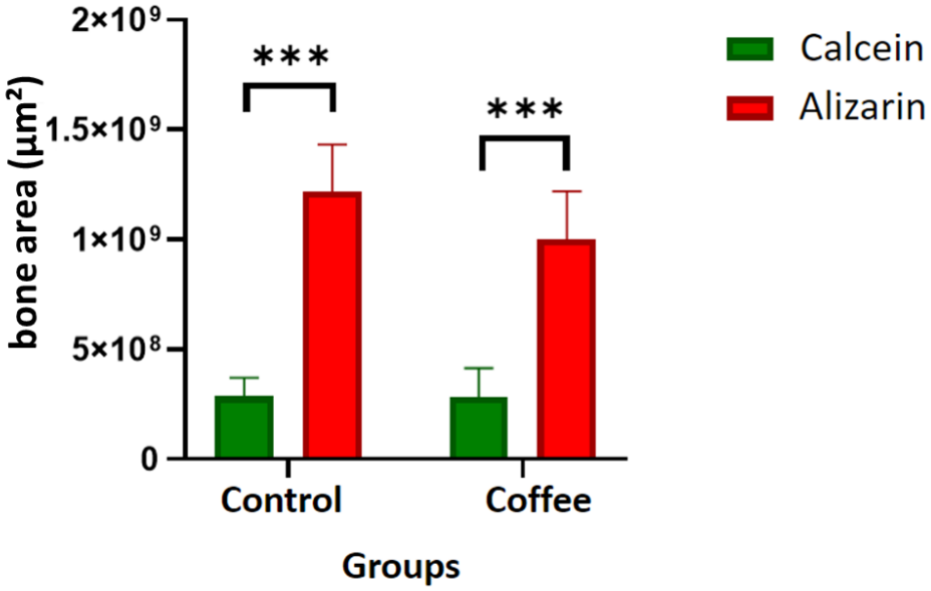

Fig. 3 Bone area (μm2) for the experimental group (Coffee) as assessed by calcein and alizarin staining. *** Tukey test $P<0.0001$ (intragroup comparisons of statistical significance).

Figure 4 showed the image of the amount of old bone and new bone. The green color (calcein) marks the older bone, whereas the alizarin marked the new bone.

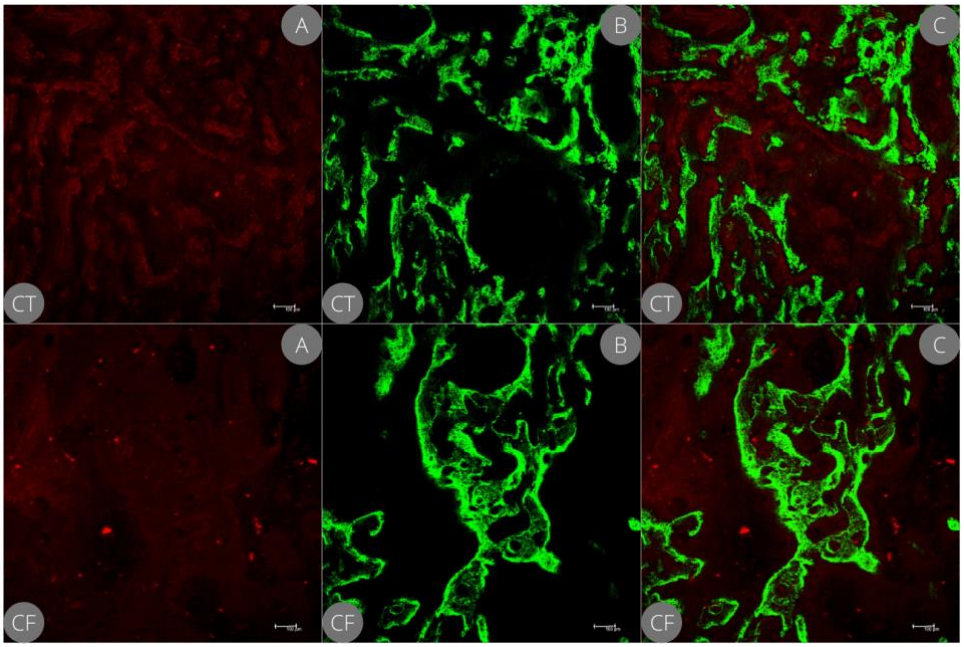

Fig. 4 Alveolar bone images obtained using confocal microscopy. CT: Control group; CF: coffee group.

Discussion

The impact and the relevance of coffee on health is based on the knowledge that it is a beverage that is consumed all over the world. It was described that coffee is the drink with the largest proportion of adults reporting consumption, after water [20]. It includes a wide array of components that can have potential implications on people's health [10, 11].

A study conducted in the Brazilian population showed that, between the years 2008 and 2009, the estimated average usual daily coffee intake was 163 mL [20]. The Brazilian Institute of Geography and Statistics (IBGE) communicated that coffee is the most daily consumed food by 79% of the Brazilian population over 10 years of age [21]. The Coffee Consumption Trends (TCC) survey carried out in 2010 showed that 97% of respondents are coffee consumers, and the main reason reported for the consumption of the beverage among the participants was the fact that it is a family habit or tradition, brought since childhood, in addition to having characteristics associated with pleasure and sociability [22].

Previous work showed that growing rats that had caffeine in their diet had decreased mineralization of the bone matrix of their tibias. Caffeine caused significant reductions in extracellular matrix production, mineralization and alkaline phosphatase activity, accompanied by decreased gene expression of cartilage-specific matrix proteins such as aggrecan, type II and type X collagen [23]. Studies have also shown detrimental effects of caffeine on bone tissue impacting calcium balance [24-27].

Fluorochromes have the property of binding to calcium in the moment that it is going to precipitate on the organic bone matrix. Thus, because of this direct relation, the extent of fluorochrome labeling represents the quantity of calcium precipitation, and consequently quantify the bone formation event [17, 28-29]. In order to obtain the mineral apposition rate in the current paper, two fluorochromes (calcein and alizarin) were injected in the rats from both control and coffee groups. At 14 days from the day of the tooth extraction, intravenous green calcein was administered, and, at 24 days from the day of the tooth extraction, intravenous alizarin red was administered. The first fluorochrome injected, calcein, indicates calcium deposition in old bone. The last injected fluorochrome, alizarin, represents the newly-formed bone, which can be called the active surface of mineralization [16-17].

In the present study, there was no statistically significant difference between groups for mineral apposition rate. Despite that, the coffee group showed a tendency to have a decrease in alizarin, i.e., the young bone is reduced. This event was explained by Zhou et al. [30], which described that the caffeine contained in coffee causes a reduction in the differentiation of mesenchymal stem cells in osteogenic lineages and inhibit some specific gene expression, thus affecting osteogenesis. It is assumed that caffeine participates in the expression regulation of the Cbfa1/Runx2 gene and decreases differentiation rate of mesenchymal stem cells in osteoblasts [12]. Samoggia and Riedel [31] reported that compounds of coffee, notably the caffeine, weaken calcium absorption and promote its excretion [31].

In the present study, the administration of fluorochromes formed fluorescent lines by calcium precipitation in the organic matrix next to calcein (Green fluorochrome given on day 14) and by calcium precipitation next to alizarin (Red fluorochrome given on day 28). Then, the green marker on the slides is the mineralization of the bone formed in the middle of the alveolar repair of both groups. As alizarin red is the fluorochrome administered at the end of alveolar bone repair, was observed red in the most recent mineralization on the slides, that is, the bone formed at the end of the repair. The decrease of alizarin in the coffee group showed the impact of coffee on calcium metabolism generates this tendency to decrease the mineralization in alveolar repair.

Since research has shown that coffee is among the most consumed foods in Brazil [32], the present study figured out about the consequences that diet can have on bony metabolic processes and how this affects alveolar bone repair. There are several post-operative recommendations for patients, including not to consume hot drinks for the first few days after surgery. However, after a short time, patients return to consume their diet normally, including coffee, which can be present in the first few days if this drink is made cold, as it is

found in some foods. This consumption then returns even before alveolar bone repair is completed. In this paper, it was investigated the effects of coffee ingestion on the alveolar bone dynamics after dental extraction in Wistar rats. Some of the previous studies [14, 33-38] that can be found in the literature aimed to analyze the influence of the caffeine in bone. That implies in different results, once the coffee includes a complex mixture of many bioactive compounds, with physiological effects, and the caffeine is just one of them. The coffee includes up to 1000 phytochemicals, as phenols including chlorogenic and caffeic acid, lactones, and others [10-12]. Another thing that must be considered is that caffeine is found in several foods and medicines consumed by the population. Thus, not only the coffee present in the diet, but also various products that are consumed can add caffeine, which, with the tendency for less mineralization found in this study, can cause tissue less mineralized after repair. Thus, analyzing the effects of the caffeine itself brings results that are different from those obtained when analyzing the effects of the coffee ingestion.

Besides that, coffee composition may vary depending on the method of its preparation. The amount of caffeine in a cup of coffee, for example, is influenced by the method of coffee preparation [10]. This can possibly explain why some results found in previous studies and the impact of coffee consumption on bone metabolism remains controversial. And this can be characterized as a limitation of this and other studies.

Conclusion

In conclusion, the effect of coffee ingestion on alveolar bone dynamics after tooth extraction was verified a tendency for there to be a decrease in renewed bone in the group that drank coffee.

Conflict of interest

The authors declare that they have no conflict of interest.

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3 CONCLUSÃO

Em conclusão, o efeito da ingestão de café sobre a dinâmica óssea alveolar após a extração dentária foi verificado uma tendência de haver diminuição do osso renovado no grupo que bebeu café.

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[1*] De acordo com as normas da UNICAMP/FOP, baseadas na padronização do International Committee of Medical Journal Editors - Vancouver Group. Abreviatura dos periódicos em conformidade com o PubMed.

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ANEXOS

Anexo 1 – Verificação de originalidade e prevenção de plágio

INFLUÊNCIA DO CAFÉ NA DINÂMICA DO REPARO ÓSSEO ALVEOLAR EM RATOS: ANÁLISE DE MICROSCOPIA CONFOCAL A LASER

RELATÓRIO DE ORIGINALIDADE

3%	2%	1%	1%
ÍNDICE DE SEMELHANÇA	FONTES DA INTERNET	PUBLICAÇÕES	DOCUMENTOS DOS ALUNOS

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Anexo 2 – Comitê de Ética em Pesquisa

UNICAMP

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CERTIFICADO

Certificamos que a proposta intitulada **Influência da cafeína na osteogênese do reparo ósseo alveolar em ratos Wistar**, registrada com o nº **5324-1/2019**, sob a responsabilidade de **Prof. Dr. Ana Cláudia Rossi e Kaio dos Santos**, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo *Chordata*, subfilo *Vertebrata* (exceto o homem) para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais, do **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**, e com as normas editadas pelo **Conselho Nacional de Controle da Experimentação Animal** (CONCEA), **tendo sido** aprovada pela **Comissão de Ética no Uso de Animais** da **Universidade Estadual de Campinas - CEUA/UNICAMP**, em reunião de **08/08/2019**.

Finalidade:	**( ) Ensino (X) Pesquisa Científica**
Vigência do projeto:	01/10/2019 a 31/07/2020
Vigência da autorização para manipulação animal:	08/08/2019 a 31/07/2020
Espécie / linhagem/ raça:	Rato heterogênico / HanUnib: WH (Wistar)
No. de animais:	4
Idade/Peso:	2.00 Meses / 250.00 Gramas
Sexo:	4 Machos
Espécie / linhagem/ raça:	Rato heterogênico / HanUnib: WH (Wistar)
No. de animais:	4
Idade/Peso:	2.00 Meses / 250.00 Gramas
Sexo:	4 Machos
Espécie / linhagem/ raça:	Rato heterogênico / HanUnib: WH (Wistar)
No. de animais:	4
Idade/Peso:	2.00 Meses / 250.00 Gramas
Sexo:	4 Machos
Espécie / linhagem/ raça:	Rato heterogênico / HanUnib: WH (Wistar)
No. de animais:	4
Idade/Peso:	2.00 Meses / 250.00 Gramas
Sexo:	4 Machos
Origem:	CEMIB-UNICAMP
Biotério onde serão mantidos os animais:	Biotério da Faculdade de Odontologia de Piracicaba, FOP/UNICAMP

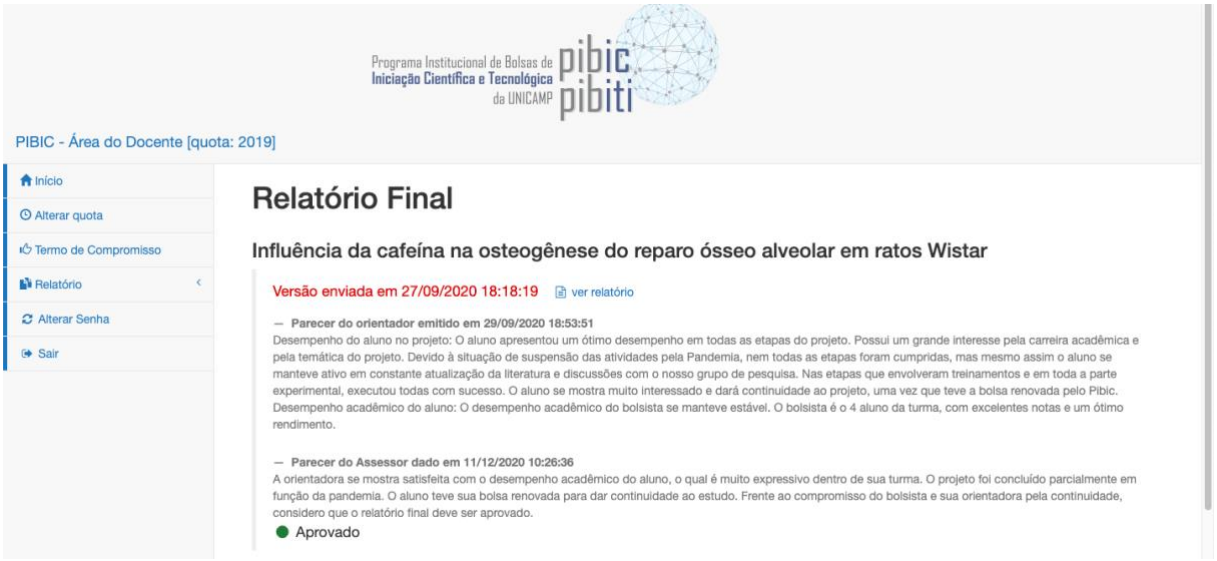
A aprovação pela CEUA/UNICAMP *não* dispensa autorização a junto ao **IBAMA**, **SISBIO** ou **CIBio** e **é restrita** a protocolos desenvolvidos em biotérios e laboratórios da Universidade Estadual de Campinas.

Campinas, **07 de novembro de 2019**.

Prof. Dr. Wagner José Fávaro
Presidente

Rosangela dos Santos
Secretária Executiva

Anexo 3 – Iniciação Científica



Anexo 4 – Comprovante de submissão do Artigo